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A Role for Photoreceptor Outer Segments in the Induction of Deprivation Myopia

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An ultrastructural examination of the outer retina and choroid of hatchling chicks reared for periods of 1, 2 or 4 weeks with opaque occluders (MD) covering one eye, was instigated to elucidate the mechanism of deprivation myopia. Refractive myopia (~20 D), retinal and choroidal thinning were induced in all deprived eyes. Electron microscopy showed significant changes in the MD eyes compared to normals. Cone inner segments were markedly thicker and outer segment lamellae more damaged. The rod outer segments were elongated and thicker than normal, such that their distal tips either directly apposed the basal lamina of the retinal pigment epithelium, or indented the cell nuclei. We hypothesize that this "rod-push" mechanism leads to thinning of the choroid in deprived eyes, and may directly contribute to axial myopia.

Myopia Deprivation Photoreceptor Electron microscopy Chicken

INTRODUCTION

The important role of visual experience in the induction of refractive errors has been established over the past 15 yr. Deprivation of form vision early in life has been shown to induce significant axial myopia in several species of vertebrates including human (Curtin, 1985), monkeys (Wiesel & Raviola, 1977), tree shrews (Norton, Essinger & McBrien, 1989) and chicks (Wallman, Turkel & Trachtman, 1978; Wallman, 1979).

Recently the chick has become a popular model for myopia research, as visual deprivation in this species leads to profound refractive myopia and axial elongation more rapidly than in other vertebrate species studied. As little as 1 week of form deprivation can result in 20 D of myopia and vitreous chamber enlargement by nearly 1 mm. The term form-deprivation myopia (FDM) has been used to cover all experiments involving occlusion-based rearing (from dark opaque to diffuse translucent occluders, and eyelid suture). While axial elongation and myopia are findings common to all these experimental paradigms, it is not yet clear whether the mechanisms involved are identical. It appears that FDM is not a response of the eyeball to changed physical conditions under an occluder, such as increased temperature (Hodos, Revzin & Kuenzel, 1987), but rather that FDM requires an intact functioning visual system. Retinally blind chicks do not develop abnormal elongation in the presence of occlusion (Oishi & Lauber, 1988). Curiously, rearing chicks in constant light also results in myopia and axial elongation (Lauber & McGinnis, 1966).

The mechanism of abnormal eye growth underlying FDM has been explored from several directions. Form deprivation has been effected via lid suture or occlusion with varying degrees of light transmission loss (see review Lauber, 1991) or via lens-induced optical defocus (Schaeffel, Glasser & Howland, 1988). The local retinal nature of the abnormal eye growth has been demonstrated in chickens raised with partial occlusion (Wallman, Gottlieb, Rajaram & Fugate-Wentzek, 1987), with optic nerve section (Troilo, Gottlieb & Wallman, 1987; Wildsoet & Pettigew, 1988) or lesion of the Edinger–Westphal nucleus (Schaeffel, Howland, Troilo & Wallman, 1990). Retinal function and integrity have also been systematically modified with pharmacological agents instilled in the vitreous chamber. This effectively allows dissection of the functional retina into primary, secondary and tertiary neural processing levels by suppressing or eliminating one or more of the synaptic pathways or neural elements with neurotoxins. Examples include glutamate analogues such as kainate (Wildsoet & Pettigrew, 1988; Ehrlich, Sattayasai & Zappia, 1990; Barrington, Sattayasai & Zappia, 1989; Barrington & Zappia, 1989) and quisqualate (Barrington & Zappia, 1989), which are known to affect second-order neurons, aminoadipic acid (Crewther & Crewther, 1990) whose isomers separately affect ON and OFF retinal light responses, and formoguanamine, a uracil analogue

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known to cause retinal blindness in hatchling chickens by damaging the outer retina (Oishi & Lauber, 1988).

Ehrlich *et al.* (1990) suggested that bipolar cells, amacrine cells or photoreceptors must be involved in FDM, on the basis of comparison of the differential effects on ocular growth of quisqualate and kainate in combination with form deprivation. The involvement of retinal ganglion cells was rejected on the basis of deprivation myopia occurring in optic nerve sectioned chicks (Troilo *et al.*, 1987; Wildsoet & Pettigew, 1988). There is also evidence of damage to photoreceptors at the doses used in the kainate studies (Ingham & Morgan, 1983) and of considerable disruption to the retinal pigment epithelium (RPE) and photoreceptors in formoguanamine-treated chicks (Oishi & Lauber, 1988; Obara, Matsuzawa, Kuba & Fujita, 1985). On the other hand, FDM has been shown to be extremely sensitive to manipulation of dopamine levels within the retina (Stone, Lin & Laties, 1989; Rudolf & Wioland, 1993; Lin, Stone, Laties & Iuvone, 1988), suggesting that the amacrine cell layer, the chief retinal location of dopamine receptors, may be implicated in the FDM process (Stone, Lin, Iuvone & Laties, 1990; Weiss & Schaeffel, 1993).

Even the way in which the retina detects and responds to optical defocus of either sign (Irving, Callender & Sivak, 1991; Schaeffel, Hagel, Kohler & Zrenner, 1992; Schaeffel *et al.*, 1990) remains a puzzle, and indeed, it has been suggested that the response to defocus may use a different mechanism from that occasioned by occlusion (Schaeffel *et al.*, 1992). One of the more obvious sources of sign-sensitive defocus detection local to the eye, chromatic aberration, has been investigated with respect to its influence on emmetropization, but without many positive findings (Wildsoet & Howland, 1991; Rohrer, Schaeffel & Zrenner, 1992; Wildsoet, Howland, Falconer & Dick, 1993).

Despite the numerous experiments which have been performed delineating the experimental conditions which lead to FDM, little detailed structural analysis of the effects on eyes or retinas of FDM has appeared in the literature (Ehrlich *et al.*, 1990; Yinon, Koslowe, Lobel, Landshman & Barishak, 1982), apart from the initial report in monkey (Raviola & Wiesel, 1982). Ehrlich has reported a 22% reduction in retinal thickness in chicks occluded for 21 days post-hatching. The thinning was most noticeable in the outer nuclear layer (26%), inner nuclear layer (33%) and inner plexiform layer (25%) (Ehrlich *et al.*, 1990). The RPE in monocularly deprived (MD) eyes was reported to be "not different from control eyes", although changes in the choroid and sclera have been reported (Christensen & Wallman, 1989; Gottlieb, Joshi & Nickla, 1990; Pickett-Seltner, Sivak & Pasternak, 1988; Reiner, Fitzgerald & Hodos, 1991). There have also been reports of disrupted lamellae in some photoreceptor outer segments (OS) (Ehrlich *et al.*, 1990). However, no description of the sequence of structural changes that occur in the neural retina in the presence of form-depriving occluders is available, although descriptions of the ultrastructure of form deprivation in association with various neurotoxins have been published (Sattayasai & Ehrlich, 1987; Sattayasai, Zappia & Ehrlich, 1989).

The chicken retina is typically vertebrate in its organization and stratification, despite the absence of retinal blood vessels and the presence of a markedly protuberant pecten. The photoreceptors, also show some differences in detail from the primate. Cone photoreceptors in chick possess oil droplets at the boundary between the inner and outer segments. There are two main cone types—single and double cones. Single cones contain a relatively large oil droplet [of two different colours (Morris & Shorey, 1967)] and double cones (not found in primates) consist of a chief cone and an accessory cone. The accessory cone has a conspicuous paraboloid. The myoid region of the chief cone contracts in the light, while the accessory cone does not possess this retinomotor capability. Throughout the chicken retina double cones are twice as numerous as single cones, and equal in number to rods in all regions except a circumscribed area posterior to the pecten (Meyer & May, 1973).

In this study, the morphology and ultrastructure of the outer retina of chicks reared with monocular occlusion using opaque occluders for periods of 1–4 weeks was compared with that from the fellow eyes receiving normal visual experience, to allow examination of the anatomical components and their relation to each other throughout the time of deprivation. A comparison of the morphology of retinal photoreceptors under the two conditions suggests a novel mechanism for FDM.

MATERIALS AND METHODS

Thirteen hatchling meat chicks (a strain of chicks developed from White Leghorn, commonly referred to as English Cobb 500) of mixed sex were raised in a thermally regulated environment of temperature 31–35°C with unlimited food and water, and a 12 hr/12 hr day–night cycle under automatic control, with light provided by a 40 W incandescent lamp. Monocular occlusion in the chicks was produced by gluing black heat-formed styrene hemispheres to the periocular feathers with cyanoacrylate glue on day 2.

Chicks were reared from day 2 after hatching with monocular occlusion for periods of 1 week (5 chicks), 2 weeks (5 chicks) and 4 weeks (3 chicks) at which time occluders were removed and the refractive state of the unanaesthetized eyes was rapidly determined using retinoscopy. The untreated fellow eyes were used as controls. Thus the following groups of eyes were established: MD 1 week, 2 week and 4 week; and NORM 1 week, 2 week and 4 week. The animals were sacrificed immediately after retinoscopy was completed.

Rapid retinoscopy was required to restrict the light exposure to the occluded eyes to less than 2 min and to minimize the large accommodative changes seen in the eyes. Animals were not anaesthetized nor was mydriatic used as the protocol demanded as little physiological or pharmacological change to the occluded condition as

possible. The profound differences in refractive status between each animal's two eyes did not make long refractive assessment necessary.

Chicks were sacrificed by barbiturate overdose and the eyes immediately injected intravitreally with fixative and then prepared for light and electron microscopic examination. The eyeballs were removed from the orbits and immersion-fixed in 2.5% glutaraldehyde, 2.5% paraformaldehyde and 2% DMSO in 0.1 M sodium cacodylate buffer for at least 1 hr at room temperature. The eyeball was opened along the ora serrata, and the eyecup was dissected into 2 mm tissue pieces from five regions (see Fig. 1). The tissues were post-fixed in 2% osmium tetroxide for 1 hr. Samples were then dehydrated and embedded in epon-araldite. Thick sections of 1 μm were cut and stained with Toluidine Blue for light microscopy (LM). Thin sections were made on an LKB ultramicrotome at 60–90 nm thickness. The sections were stained with 4% uranyl acetate and 2% lead citrate and examined in transmission electron microscopy on a Hitachi-7000 electron microscope. Care was taken to sample tissue for comparison from corresponding locations of the retinas.

Retinal layer thicknesses were measured from the five regions of all chicks using the LM sections. High power LM photographs were used for photoreceptor OS length measurements and for RPE cell dimensions. Estimation of mean minimal distance of the photoreceptors from Bruch's membrane was accomplished by calculating the mean minimal approach of the photoreceptor OS to the basal lamina of the RPE from a series of photographs, each of which covered 350 μm of lineal extent of Bruch's membrane.

The animals were raised in accordance with the regulations of the National Health and Medical Research Council of Australia, and the conditions employed conform to the NIH document "Guiding Principles in the Care and Use of Animals".

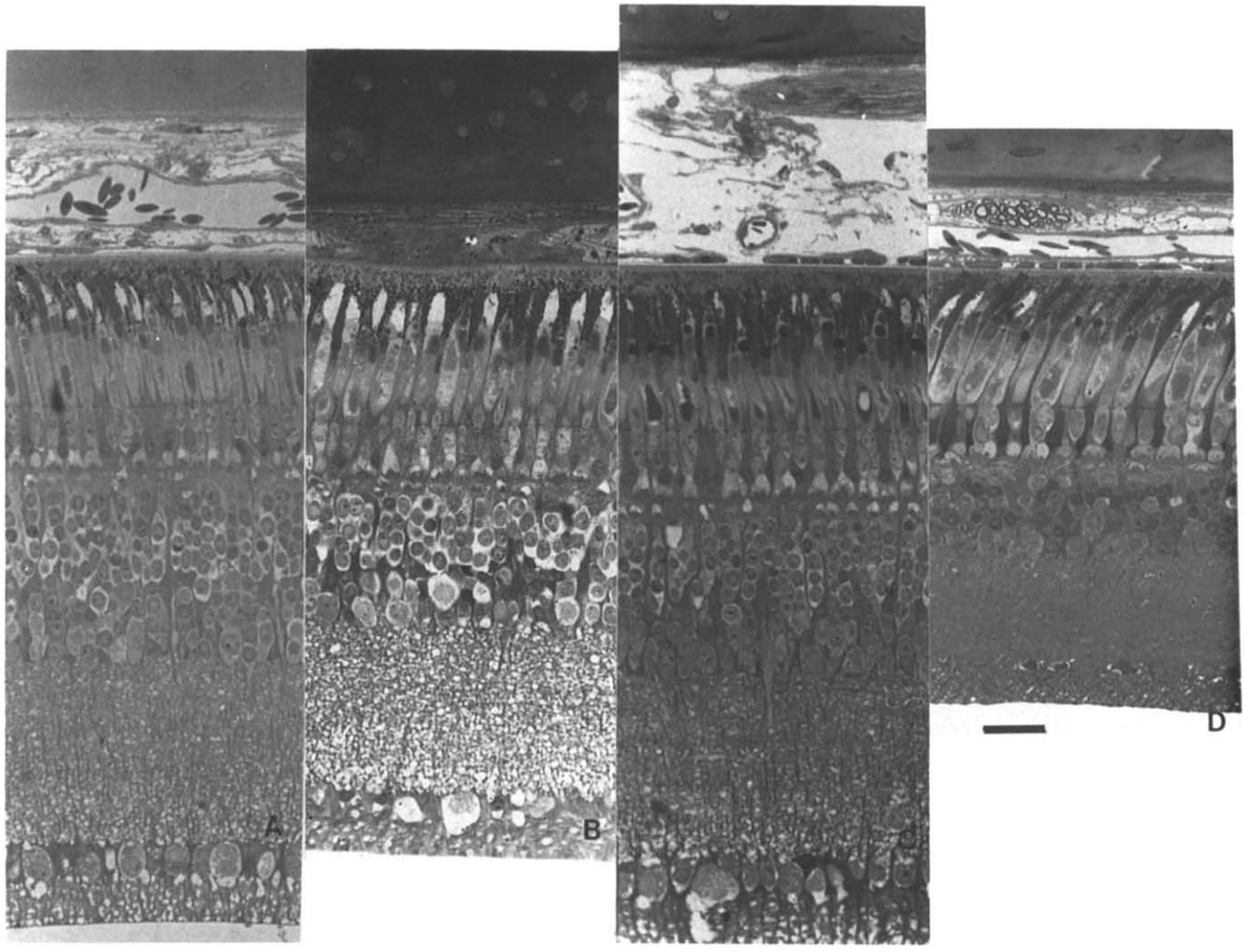

FIGURE 1. Light microscopy of normal and MD retinas at ages 1 and 2 weeks (transverse sections stained with Toluidine Blue). The sections have been aligned along Brcuh's membrane. (A) 1 week NORM. Note the open vessels of the choroid. (B) 1 week MD. The choroid is occluded. Considerable swelling in the innermost layer of amacrine cells is apparent. (C) 2 week NORM. The choroid is thicker than the 1 week normal. (D) 2 week MD. Note the increase in the thickness of the cone inner segments and the elongation of the rod OSs such that they touch the basal lamina of the RPE. Again the choroid is very thin compared with the normal eye. Thinning of the retinas of the deprived eyes (B, D) is obvious, particularly in the inner nuclear and inner plexiform layers. The number of layered somata (in the radial direction) in the inner nuclear layer of occluded eyes was markedly less than for the normal eyes. Thinning is less pronounced in the photoreceptor layer. Scale bar represents 25 μm.

RESULTS

Refractive changes

The eyes which wore occluders rapidly became myopic. One week after occlusion was initiated, myopia of nearly 20 D was present in all of the occluded eyes compared with mild hyperopia in the fellow unoccluded eyes (see Table 1). Over the following 3 weeks, myopia did not progress significantly, although the eyeballs continued to grow and maintain the same relative axial length differential.

Light microscopy

At the light microscopic level, the immediate observation was of overall thinning of the deprived retinas and choroids compared with the normally-reared eyes (see Fig. 1 which compares transverse sections of occluded and non-occluded retinas at 1 and 4 weeks).

Following 4 weeks of deprivation, the retinal thinning, averaged over the five sampling areas, amounted to 27%. The thinning of occluded retinas relative to the age-matched controls was not confined to any particular region of the retina. However the degree of thinning varied from 18% in superior retina to 39% in the inferior retina (see Fig. 2), and the differences between deprived and normally-reared retinas were significant at the $P = 0.05$ level for all but one region (region 2, where the significance was 0.07).

Comparison of layer thicknesses between the MD and NORM retinas at 4 weeks showed a reduction in the distance from Bruch's membrane to the outer limiting membrane of 23% (measured along the orthogonal direction between these layers, or 17% measured along the alignment of the photoreceptors), while the thickness of the inner plexiform layer was reduced by 36%. The number of somata (in the radial direction) in the inner nuclear layer of occluded eyes was markedly less (6–7 neurons) than for the normal eyes (9–10 neurons see Fig. 1). There was also a qualitative tendency to smaller somata for retinal ganglion cells in the 4 week MD eyes than those of the fellow normal eyes.

The choroid was markedly thinner (up to 60% thinner) in all deprived groups and the lumens of the larger blood vessels appeared to be substantially occluded. The scleras of the MD eyes were thickened, with an increased cellular matrix component, larger cells, but decreased density.

TABLE 1. Refractive status

Age	Refractive state: NORM (D)	Refractive state: MD (D)
1 week ($n = 5$)	$+0.4 \pm 0.25$	-19.0 ± 0.8
2 week ($n = 5$)	$+1.8 \pm 1.0$	-24.0 ± 2.9
4 week ($n = 3$)	$+0.8 \pm 0.6$	-17.5 ± 2.5

Mean refractive states in dioptres as determined by retinoscopy of the normal (NORM) and monocularly occluded (MD) eye groups used in the study. The results are presented as means ± 1 SE. Numbers of animals in the groups are shown. One week of monocular deprivation was sufficient to induce a profound myopia of nearly 20 D. This was maintained throughout the 4 weeks of occlusion.

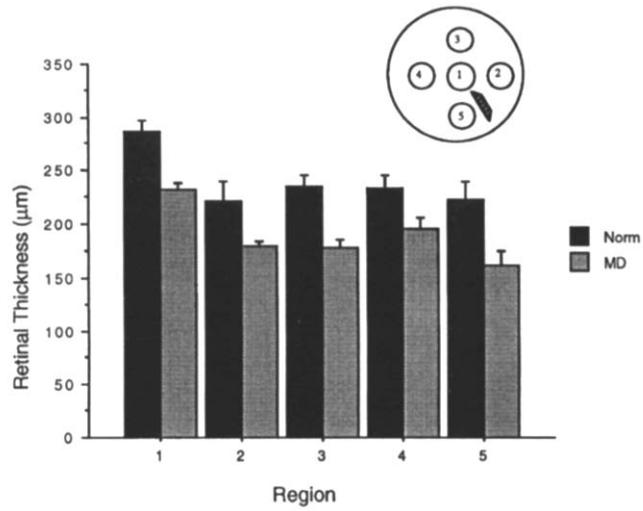

FIGURE 2. Histogram of retinal thickness measurements in the five regions studied in the MD and normal groups at 4 weeks. There was a mean shrinkage of 27% (min 18%, max 39%). The sampling strategy is indicated in the upper right portion ofthe figure, showing the five regions on a right eye (with the shaded area representing the pecten).

The issue of total scleral chondrocyte number has not been addressed.

Despite the general trend of retinal thinning [areal enlargement of the retina presumably resulting from increased vitreous chamber depth and equatorial dimensions (Wallman *et al.*, 1978; Hodos & Kuenzel, 1984)], the photoreceptors appear to respond differently to deprivation. A decrease in photoreceptor density seems apparent, with a thickening of the cone photoreceptor inner segments. However, this was offset to some degree by a thinning and elongation of the rod inner segments. The accessory cone type appears particularly prominent in the MD retinas owing to considerable swelling of the inner segment and characteristic staining of the paraboloid. Also, the overall photoreceptor length was not as greatly reduced in the occluded retinas relative to eyes receiving visual experience. The thickening of the cone inner segments gives an immediate impression of a marked reduction in photoreceptor density.

The lengths of the photoreceptor OS were measured from 896 rods and 581 cones from MD and NORM chicks at ages of 1, 2 and 4 weeks. The retinal region sampled was from the inferior mid-peripheral button of tissue (region 5) as this region showed the maximal relative thinning. A mid-peripheral region was also chosen to avoid the potentially rapid spatial variation in retinal properties expected around the area centralis (contained in region 1).

Measurements of OS length over the 4 weeks indicated that the pattern of rod OS growth in normal eyes in the first 2 weeks is characterized by rapid elongation through addition of extra lamellae. This is followed by a small but significant reduction in length at a little less than 40 μm. By comparison both 1 and 2 weeks of deprivation resulted in rod OSs which were much longer than those in the fellow eyes. The comparison at 2 weeks showed the mean rod OS length to be 52 μm for the MD eyes compared with 43 μm for the normal eyes (difference significant at the $P = 0.0001$ level,

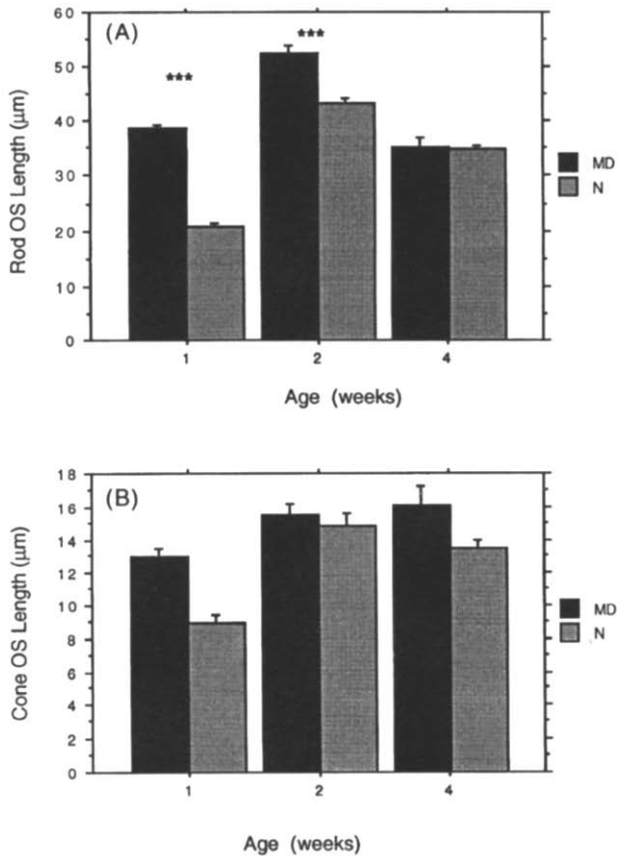

FIGURE 3. Histogram of photoreceptor OS lengths measured from inferior mid-peripheral retina of normally-reared and occluded eyes. (A) Rods OS lengths. Normal OS show an increase in length until at least 4 weeks post-hatching. OSs for the 1 and 2 week MD eyes show a very rapid increase in OS length. However, by 4 weeks the OS lengths are insignificantly different from those in normally reared eyes. (B) Cone OS lengths also show an increase with age. Occlusion also caused an increase in OS length, although not with the same level of significance as observed with the rod OS. ***Significance at the $P = 0.0001$ level.

Student's t-test). However, after 4 weeks of MD, the rod OSs of MD eyes were insignificantly different in length from those of fellow normal eyes (see Fig. 3).

This rod photoreceptor OS elongation resulting from deprivation is correlated with a drastic reduction in the distance from the tips of the OSs to the inner margin of Bruch's membrane (see Fig. 4). The cone OSs in normal

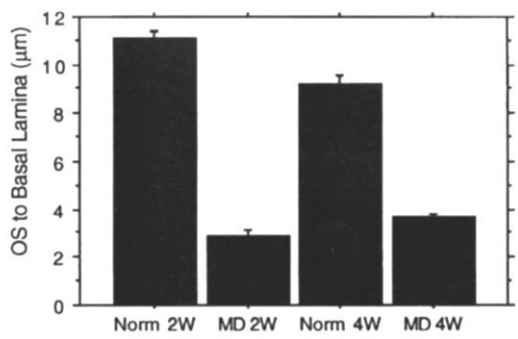

FIGURE 4. The mean minimum distance from the tips of photoreceptor OS to Bruch's membrane as measured in a series of 186 photographs, each covering a linear distance of 350 μm along Bruch's membrane.

chicks possess an early phase of membrane addition similar to that seen for the rods. Comparison of MD with NORM groups shows that while at 1 wk MD cone OSs were larger, for the two later ages cone OS means ranged between 13 and 16 μm without significant differences (Fig. 3).

The histogram of Fig. 4 shows the mean minimum distance of approach of the photoreceptors to Bruch's membrane assessed from a standard number of LM photographs, each covering 350 μm along Bruch's membrane.

Electron microscopy

Under electron microscopy the deleterious effect of deprivation on the outer retina is much more apparent. Particularly noticeable was the disruption and degeneration of the cone OS membranes in the MD retinas. This damage becomes more extensive and severe with the period of occlusion. Following 4 weeks of occlusion, the regular membrane infoldings comprising the OS were almost completely absent in some cones (see Fig. 5). The chief cones of the double cone pair seem the most susceptible to damage. The OS of the thinner single cone and the accessory cone OSs appear to survive better. The lamellae of the elongated rod OSs appear to be intact, with apparently unchanged spatial density, indicating a greatly increased number of lamellae per OS compared with normals. For the 4 week MD retinas, many rod OSs actually touch the RPE basal lamina (see Fig. 5). Also, the rod OSs were much fatter in the MD compared with the normal eyes.

The nuclei of RPE of the MD retinas were often indented, presumably by the photoreceptor OSs. In many instances the OS of a rod or of an accessory cone is found dovetailed into the RPE nucleus. It is interesting to note that in the 1 week NORM group, the OSs are quite close to the basal membrane but that the distance between the outer limit of the rod OS, and the basal membrane of the RPE increases with age. In these normal eyes, there is a much denser layer of mitochondria lying between the OS and the basal lamina than in 1 week MD eyes, and the impacting of OS on RPE nuclei although occasionally present, is not common nor severe. Similarly, in the 2 and 4 week NORM groups there were always more mitochondria in a layer next to the basal lamina of the RPE than in the corresponding MD groups.

A closer inspection of the RPE nuclei of the MD group shows that most have an irregular membranous perimeter, compared with the smooth oval outlines of RPE nuclei in the NORM group (see Fig. 5). The irregularities observed in RPE nuclei and the location of the rod OSs against the basal lamina of the RPE present strong evidence for multiple impacts of the OSs on the basal RPE lamina during a period of deprivation. The linear density of RPE cells was reduced in the deprived eyes, indicative of a stretching of the tunic of the enlarged eyes (see Table 2).

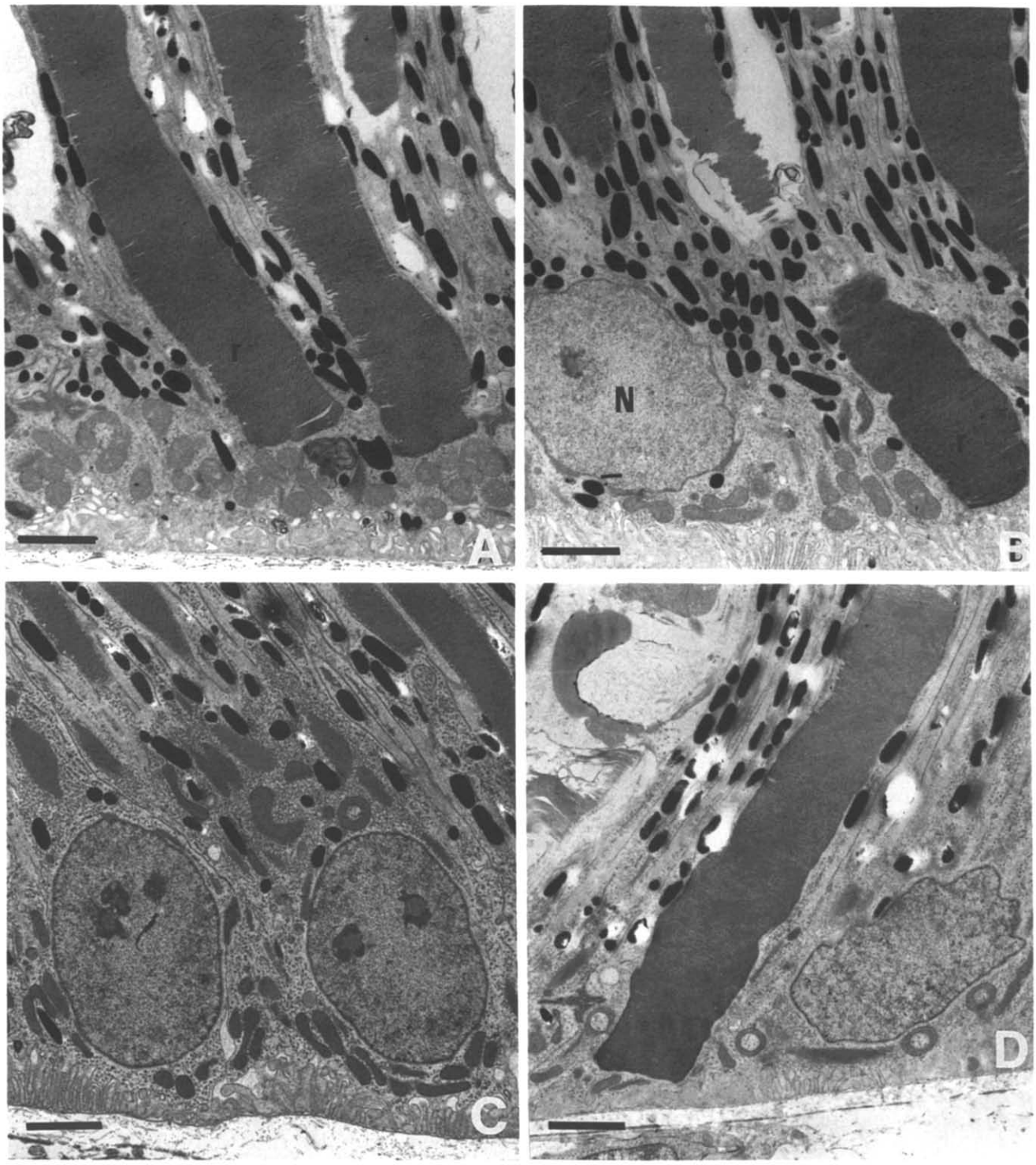

FIGURE 5. Electron microscopy of 1 and 4 week NORM and MD outer retina. (A) NORM 1 week retina showing two rods (r) as well as numerous mitochondria lying between the OS and the basal lamina of the RPE. (B) Rod OS from 1 week MD chick showing a rod OS (r) almost reaching the basal lamina. (C) NORM 4 week. Notice the smooth profile of the RPE cell nuclei (n) and the distance of the rod and cone photoreceptor from the basal lamina of the RPE cell. (D) MD 4 week. The rod OS are quite elongated, much thicker and extend more deeply into the RPE cell than those in (C). The rod is actually depressing the basal lamina of the RPE cell. Note also the characteristic reduction in the total number of mitochondria to be seen and the lowered density and height of the basal laminar membrane in the RPE cell. There is also a relative increase in the number of ring-shaped mitochondria compared to the number of normal circular or dumbell-shaped profiles. Note also the degeneration of the OS lamellae and oil droplet of the cone in the upper left corner. The nucleus is also much more indented and less healthy looking than the comparable smooth elliptical deeply staining nucleus of the normal 4 week RPE cell in (C). The scale bar indicates 2 μm.

The disposition of melanin granules in the RPE cells under occluded and normally-reared conditions also showed some differences. In normally-reared chick, the melanin granules migrate vitreally during daylight and migrate sclerally during darkness. In the deprived eyes, melanin granules are uncharacteristically dispersed

TABLE 2. Comparison of outer segment lengths

Group	MD (μm)	NORM (μm)	P-value
Rod outer segments			
Week 1	38.5 ± 5.4	20.8 ± 0.7	<0.0001
Week 2	52.4 ± 1.3	43.4 ± 0.9	<0.0001
Week 4	35.1 ± 1.7	34.9 ± 0.4	0.850
Cone outer segments			
Week 1	13.0 ± 0.4	8.9 ± 0.5	0.09
Week 2	15.5 ± 0.7	14.8 ± 0.8	0.55
Week 4	16.1 ± 1.1	13.5 ± 0.5	0.06

Comparison of 1477 photoreceptor OSs measured in the normally reared and deprived eyes from 1, 2 and 4 week old chicks. The results are presented as means ± 1 SE. The significance of the comparison between MD and NORM eyes is indicated in the right column.

alongside the photoreceptor OS, despite the constantly darkened environment under the opaque occluders used in this experiment.

DISCUSSION

The most significant structural difference between the outer retinas of normal and deprived myopic eyes was the excessive elongation of photoreceptor OSs observed in occluded eyes and the extremely close apposition of the photoreceptor OSs to the nuclei and basal laminae of the RPE cells which resulted from this elongation. That active force is involved in this apposition is indicated by the frequency of occurrence and depth of indentations that were commonly observed in the RPE nuclei of the MD retinas. The RPE nuclei of the fellow normally-reared eyes were normally smooth in profile, giving no indication of pressure being exerted by photoreceptors. The depth of indentation of RPE nuclei seen in many sections also gives an indication of the relative hardness or rigidity of OSs compared with other tissue elements.

Support for the idea that photoreceptor length and axial elongation are intimately related comes from the pattern of growth observed in deprived eyes. The growth rate in deprived eyes appears to be considerably faster in the first 2 weeks of occlusion than in successive weeks (Crewther & Crewther, 1990). This correlates with the resolution of rod OS length differences between MD and NORM groups by 4 weeks. Also, the local nature of myopia induction (based on myopia in optic nerve section chicks and local FDM in partial occlusion experiments), would be compatible with the local nature of the retinomotor control in chick and other species. Also, the association between photoreceptor OS elongation and myopia is supported by studies of chicks reared in constant light [the light induced avian glaucoma syndrome (Lauber, Shutze & McGinnis, 1961)]. An elongation of frog rod OSs is also observed in continuous darkness (Basinger, Hoffman & Matthes, 1976) and even more strikingly in continuous light (Currie, Hollyfield & Rayborn, 1978), where the rod OSs increased in length by 59%.

It is curious that under the lighting environment imposed by the occlusion used, the expected contraction of the rod myoids does not appear to occur. Rather, the rod myoids appear, if anything, to be elongated.

The observation of greatly reduced distance between the tips of the OS of the photoreceptors and the RPE basal lamina can provide a hypothesis for the mechanism of FDM, namely, that deprivation produces an elongation of the photoreceptor OSs and exerts pressure on the basal lamina of the RPE. This pressure could contribute to the reported choroidal thickness reduction and collapse of the choroidal vessels observed, with the consequent reduction in blood flow in FDM eyes (Reiner *et al.*, 1991). As short a time as 1 week of form deprivation was sufficient to cause the collapse of all the major choroidal vessels. The implied lessening of blood flow would almost certainly affect the delivery of oxygen to the posterior retina. While lessened blood flow does not automatically prejudice oxygen sufficiency, the demand would be increased by the dark currents occasioned by the black opaque occluder used.

Elongation of the OSs is not the only possible mechanism for exerting force on the RPE. The myoids of the photoreceptors in a large number of vertebrate species, including the chicken, have the ability to shorten or elongate in response to illumination and in coordination with the movement of pigment that surrounds the photoreceptors. In the light, the rod myoids elongate, burying their more sensitive OS in the RPE and the cone myoids contract, moving the OSs closer to the incoming light, except for the accessory member of the double cone which does not contract (Cheng, Shoffer, Gelatt, Gum, Otis & Bitgood, 1980; Fite, Montgomery, Whitney, Boissy & Smyth, 1989). Correspondingly, pigment granules migrate into the microvilli of the RPE cells, effectively shielding the rods from excessive luminance levels. Opposite reactions occur in the dark (Bok, 1985). These retinomotor movements occur on a local scale within the retina.

Two features emerge. In these chicks, the MD eye has experienced a low-light form-deprived environment. It is curious and perhaps highly significant that the photoreceptoral elements chiefly involved in pushing against the basal lamina of the RPE are rod photoreceptors; i.e. in the MD eyes (in relative darkness) the rods are extended, rather than retracted. The second is that the local nature of retinomotor movements and the involvement of photoreceptors in myopia are compatible with the local nature of myopia, observed in partial occlusion experiments (Wallman *et al.*, 1987). Retinomotor movements also occur in response to dopamine and its agonists (Pierce & Besharse, 1985).

It is difficult to assess the relative contribution of the rods and cones to this proposed mechanism for eye growth control. Certainly the cone OSs show initial relative elongation at the 1 week stage. However, their greater sensitivity to the altered environment in the deprived state results in much greater degeneration and disorganization of the lamellae of the OS. This degeneration must eventually limit their capacity to be involved in the exertion of pressure on the outer layers of the retina.

The changes that occur in the thickness of the choroid during occlusion and following its removal have been well documented. From these observations, Wallman *et al.* have suggested that the swelling of the choroid observed following the removal of an occluder helps to push the retina back towards emmetropia (Wallman, Wildsoet, Krebs, Gottlieb, Marran & Nickla, 1992). Given the anatomical evidence of our study, a mechanism for the induction of growth and the thinning of the choroid in MD animals is proposed. The extension of the photoreceptors, particularly the rod photoreceptors, through a combination of OS elongation and retinomotor "push" has resulted in pressure on the RPE, which is transferred to the choroid to the extent that total occlusion of the large choroidal vessels occurs. Presumably, this pressure is also transmitted to the sclera. Thus, conditions for stretch or induced growth of the outer tunic of the eye are present.

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